PATENT COOPERATION TREATY

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Rec'd PCT/PTO 30 NOV 2004

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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30-11-04 (9um) / nat. phase 2

23 SEP 2004

BEANWOORD

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	23.09.2004
Applicant's or agent's file reference R60491PC00	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL 03/00408	30.05.2003	31.05.2002

Applicant
KIADIS B.V. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:
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PATENT COOPERATION TREATY

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Rec'd PCT/PTO 30 NOV 2004

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P60491PC00	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/NL 03/00408	International filing date *(day/month/year)* 30.05.2003	Priority date *(day/month/year)* 31.05.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/00		
Applicant KIADIS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29.12.2003	23.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Tuynman, A Telephone No. +31 70 340-3741

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL 03/00408

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-32	as originally filed

Claims, Numbers

1-11, 13	as originally filed
12	received on 01.09.2004 with letter of 31.08.2004

Drawings, Sheets

1/8-8/8	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 13 (in part)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 13 (in part) are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☒ the claims, or said claims Nos. 13 (in part) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 13 (in part)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/ or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-12 (fully), 13 (partially) .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	13
Inventive step (IS)	Yes:	Claims	1-12
	No:	Claims	13
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Present claim 13 relate to a compound defined by reference to a desirable characteristic or property, namely being detectable by a the method of any of claims 1-12.

The claim covers all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 6 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that the lack is not allowable according to Articles 5 and 6 PCT. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved.

An opinion on novelty , inventive step and industrial applicability shall therefore only be given for those compounds that fulfill the requirements of Articles 5 and 6 PCT, namely: phosphorylated malantide and phosphorylated kemptide and Z-FR-AMC.

Re Item IV

Lack of unity of invention

The present application does not meet the requirements of Article 17(3) PCT, because the subject-matter of independent claims 1 and 13 do not involve the same or corresponding special technical features nor are they linked by a single general inventive concept in the sense of Rules 13.1 and 13.2 PCT.

2.1 The technical features of claim 1 are the following:

a) an online detection
b) an effluent of a fractionation step is contacted with an enzyme and
c) subsequently with a substrate
d) the unreacted substrate or a modified substrate product is detected with a mass-spectrometer

The technical features of claim 13 are the compounds phosphorylated malantide, phosphorylated kemptide and Z-FR-AMC.

The technical features of claim 1 relate to the detection of compounds in an online detection system. Neither the same nor a corresponding technical feature is present in the compounds phosphorylated malantide, phosphorylated kemptide and Z-FR-AMC of claim 13. No manufacturing relationship exists between the detection method and the claimed compounds. Further the detection method is not a method of using the claimed compounds phosphorylated malantide, phosphorylated kemptide and Z-FR-AMC. There is therefore no single general concept that links the compounds to the methods. Thus unity of invention is lacking a priori.

No other technical features could be identified that form a technical relationship among the each of the separate independent claims and which could be considered as a special technical feature within the meaning of Rule 13.2 PCT.

Therefore this international preliminary examination authority considers that there are two groups of subjects of which the novelty, inventive step and industrial applicability will be assessed independently, as unity of invention is lacking among them. These two groups are as follows:

1 Claims 1-12: Mass spectrometry coupled on-line detection method of enzyme activity in an effluent of a fractionation step by detection of unreacted substrate or modified substrate product.

2 Claim 13: The compounds phosphorylated malantide, phosphorylated kemptide and Z-FR-AMC.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: INGKANINAN K ET AL: JOURNAL OF CHROMATOGRAPHY, vol. 872, no. 1-2, March 2000 (2000-03), pages 61-73.
D2: LANGRIDGE JAMES I ET AL: RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 7, no. 4, 1993, pages 293-303.
D3: WO 02 37111 A (IRTH HUBERTUS ;VRIJE UNIVERSITEIT VAN AMSTERD

(NL)) 10 May 2002 (2002-05-10)

D4: CRAIG A GREY ET AL: BIOLOGICAL MASS SPECTROMETRY, vol. 23, no. 8, 1994, pages 519-528.

D5: MURRAY K J ET AL: BIOCHEMICAL JOURNAL, PORTLAND PRESS, LONDON, GB, vol. 267, 1 May 1990 (1990-05-01), pages 703-708.

D6: KHOURI H E ET AL: BIOCHEMICAL JOURNAL, vol. 275, no. 3, 1991, pages 751-758.

1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 13 is neither new in the sense of Article 33(2) PCT nor inventive in the sense of Article 33(2) PCT.

With regard to the three compounds phosphorylated malantide and phosphorylated kemptide and Z-FR-AMC, that were mentioned in the present application, these are manifestly not novel in the sense of Article 33(2) PCT since they have been disclosed in D4 (abstract), D5 (abstract) and D6 (abstract), respectively.

2 Claims 1-12 meet the requirements of Article 33(2) and (3) PCT, because their subject-matter is novel and involves an inventive step.

2.1 D1 is considered to be the closest state of the art and discloses an on-line detection method for inhibitors of enzymes and their inhibition activity, comprising the steps of: contacting a fractionation effluent with a controlled amount of an enzyme; allowing the enzyme to interact with analytes suspected to be present in the fractionation effluent; addition of a controlled amount of a substrate for said enzyme; allowing a reaction of the enzyme with the substrate providing one or more modified substrate products; and detection of unreacted substrate or a modified substrate product using an UV-Vis spectrometer and detection of the inhibitor using a mass spectrometer (D1, abstract; page 64, figure 1c; page 67, left-hand column, line 7-page 70, right-hand column, line 3).

The subject matter of independent claim 1 differs in that the enzyme activity is measured using a mass spectrometer.
The technical effect thereof is that the activity can be measured more sensitively and that the choice of substrate is not limited.

The problem to be solved by the present invention may therefore be regarded as

providing a sensitive on-line enzyme activity detection method for any kind of enzyme substrate. The proposed solution to this problem is the detection of substrate products using mass spectrometry

This solution is not obvious from other prior art documents. For instance D2 discloses an online enzyme assay measuring substrate products with a mass spectrometer (D2, abstract; page 293, right-hand column, line 5-page 294, left hand column, line 13; page 300, right-hand column, 3rd paragraph-page 302, 1st paragraph). A frit containing the reaction mixture is on-line coupled via a continuous flow with the mass spectrometer (page 293, right-hand column, line 40-page 294, left-hand column, line 13). However, this methodology is unsuitable to be coupled to an effluent of a fractionation step as in the present invention, since the data are scanned at such a low scan rate that complete fractions would be missed. Other prior art such as D3 do relate to the coupling of an effluent of a fractionation step to a mass spectrometer but come from a technical field (receptor ligand interactions) which is too unrelated to consider their application in the field of enzymatic substrate conversion.

The skilled person would therefore not regard it as a normal design option to include this feature in the method described in document D1 in order to solve the problem posed.

2.2 Claims 2-12 are dependent on claim 1 and hence also fulfill the requirements of Articles 33(2) and (3) PCT.

3 The application does not meet the requirements of Article 6 PCT, because independent claim 1 is not clear.

3.1 The term "analytes" in claim 1 is not clear from the wording of the claim alone (GC III, 4.1). Moreover, a claim should be read giving the words the meaning they normally have in the relevant art (GC III, 4.2). According to the description (page 7, lines 25-29) an "analyte" has a very specific meaning (an inhibitor or an enzyme modifying compound) very different from the one it normally has in the relevant art, i.e. a compound which is analysed. In the present application it is the change in enzyme activity which is analysed, not the "analyte". The term analyte should therefore be replaced with a more appropriate term.

4 Claims 1-12 and 13 -in as far as claim 13 relates to the compounds mentioned in

item III- are considered to be industrially applicable in the sense of Article 33(4) PCT.

Amended claim 12

12. On-line detection method according to claim 1, wherein a mass spectrometer with a multiple-inlet unit is used, to which multiple-inlet unit different fractionation lines are connected, wherein each fractionation line comprises an effluent to which controlled amounts of enzyme and known substrates are added as described in each of the previous claims.